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Triple co-culture cell model as an *in vitro* model for oral particulate vaccine systems

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INTRODUCTION

In vaccines, proteins are often utilized as the antigen, although these are generally poorly immunogenic. To overcome this, the antigen is incorporated into a particulate system, often together with adjuvants. More specifically, lipid-based particulates such as cubosomes have been proven useful as a potential carrier for vaccines [Rizwan et al., 2013]. Cubosomes consist of a highly twisted, continuous lipid bilayer and two congruent, non-intersecting water channels, providing both hydrophilic and hydrophobic domains and a large surface area for association of antigens [Rizwan et al., 2011].

Cell culture-based *in vitro* models are a very important alternative and supplement to animal testing, and combining various cell types together in one model can improve the correlation with *in vivo* studies [Susewind et al., 2015]. A triple co-culture cell model of Caco-2 cells, dendritic cells and macrophages (Figure 1), has previously been developed for studying intestinal permeability in a state of inflammation [Leonard et al., 2010], [Susewind et al., 2015].

The aim of this study was to investigate the applicability of this cell model for testing the immunostimulatory ability of particulate vaccine formulations designed for oral delivery. Levels of cytokine production in response to vaccine administration were measured following particulate vaccine administration, as an indication of dendritic cell and macrophage activation. Cubosomes containing the model antigen ovalbumin was spray dried to obtain a particulate vaccine model system for testing in the cell model.

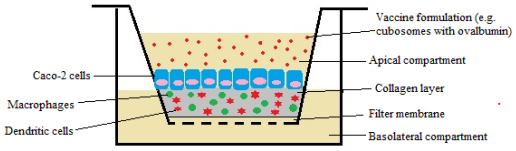

Figure 1: Schematic of the triple co-culture model being adapted for oral vaccine delivery.

EXPERIMENTAL

THP-1 cells were differentiated to macrophages, and MUTZ-3 cells grew dendritic cell-like characteristics. The cells were pre-stained and added to a solution of bovine collagen with human serum and Roswell Park Memorial Institute medium. The solution was pipetted into the apical compartment of a transwell filter insert, and incubated until the collagen layer solidified. Subsequently, Caco-2 (clone HTB37) cells in medium were seeded on top of the collagen layer. Medium was added to the basolateral compartment, and the co-cultures were grown for approximately 11 days (Figure 1). Transepithelial electrical resistance (TEER) was measured in each well during the 11 days of growth.

Precursors of cubosomes, consisting of the lipid dimodan, loaded with ovalbumin as a model antigen and with a surrounding dextran shell were prepared by spray drying. Dimodan and rhodamine dissolved in ethanol (1.78 w/v%) was first mixed with a solution of ovalbumin in water (0.075 final w/w% of ovalbumin). After 1h of mixing, dextran dissolved in water (1.77 w/v%) was added to the lipid/ovalbumin solution (0.72 w/w% of lipid + ovalbumin), and the final solution was spray dried on a Büchi mini spray dryer (Figure 2). Cryo-TEM was utilized to identify the cubic phase of the spray dried product. In addition, SAXS experiments were performed on a SAXS/WAXS beamline at the Australian Synchrotron. Hydrated cubosomes were

enclosed in glass capillaries and 2D SAXS patterns were collected.

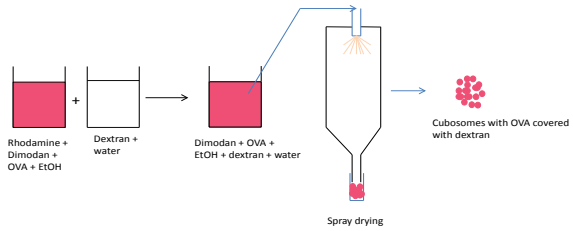

Figure 2: Spray drying of precursors of cubosomes containing ovalbumin.

On the day of experiments, vaccine formulations (Table 1) were added to the apical side of co-cultures and incubated at 37°C. After 4 and 24 hours, samples were taken out of the apical compartment and stored at -80°C until further analysis. After 24 hours (end of study), the cells were fixed and the Caco-2 cells were stained. The co-cultures were investigated under a confocal laser scanning microscope. The apical samples were analysed using a multi-analyte ELISArray kit to screen for produced cytokines.

Formulation	Concentration
Blank (only buffer)	-
Ovalbumin solution	500 µg/mL
Blank cubosomes	10 mg of dry particles
Cubosomes with ovalbumin	5 mg of dry particles
Lipopolysaccharide (LPS)	10 µg/mL

Table 1: Formulations to be tested on the co-culture model.

RESULTS

The precursors were shown to form cubosomes when dispersed in aqueous medium (Figure 3), and was therefore used as the vaccine formulation for testing on the co-cultures.

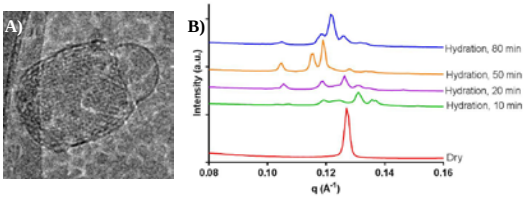

Figure 3: A) Cryo-TEM results confirmed the cubic structure of the particles. B) SAXS patterns also verified the cubic structure.

After 11 days, the TEER values of the co-cultures were found to be 860-1340 $\Omega \cdot cm^2$; the formulations were incubated with the co-cultures at this time point. From the confocal microscopy images (Figure 4), it can be observed that the THP-1 cells (macrophages – green cells) migrated into the overlying Caco-2 cell monolayer when the co-cultures were incubated with particle formulations. This was not the case when incubating with ovalbumin solution or blank.

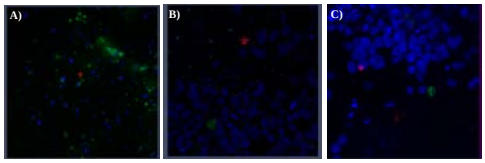

Figure 4: Confocal microscopy images of the co-cultures with Caco-2 cell nuclei in blue, THP-1 cells in green and MUTZ-3 cells in dark red. Images: A): OVA cubosomes 5 mg, 4h B): LPS 10 ug/mL 4h, C): 24 h blank sample

The ELISA screening assay showed production of a wide range of cytokines following culture incubation with cubosomes (with and without ovalbumin) and LPS solutions, indicative of a stimulatory effect; this was not observed with ovalbumin and blank solution. Example results are shown in Figure 5 for IL-2 and IL-17A.

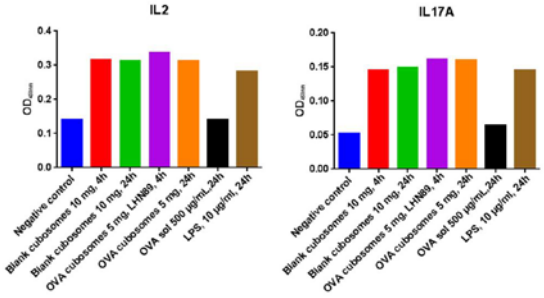

Figure 5: Examples of cytokine production of IL-2 and IL-17A after incubation of formulation with the co-cultures for 4 or 24h.

CONCLUSION

An established co-culture of Caco-2, THP-1 and MUTZ-3 cells showed promise as an *in vitro* model for testing of oral vaccine formulations. Mobility of co-culture immune cells as well as cytokine production observed following treatment with spray dried cubosomes as a particulate vaccine formulation will be further investigated.

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